

INTRODUCTION

Conifers growing at alpine and subalpine habitats are disproportionately affected by climatic changes. Increasing temperatures are reducing suitable growing habitat and impacting regeneration patterns as snowpack timing is altered. In addition to these direct effects, tree survival at high elevations is indirectly being affected by recent shifts in thermal regimes favoring biotic disturbance agents including bark beetles. Development of strategies for conservation and protection of high-elevation conifer species therefore requires an understanding of their vulnerability to bark beetle-caused mortality. Many high-elevation pine species, for example, have become susceptible to mortality due to the mountain pine beetle (MPB) (*Dendroctonus ponderosae*, Coleoptera: Curculionidae, Scolytinae) as favorable thermal conditions for population success have increased in recent decades (Bentz and others 2014). The mountain pine beetle is a native bark beetle found across western North America and is among the most important tree mortality agents in pine forests. The majority of pine species that grow in the Western United States are known hosts, although some species appear to be less vulnerable to attack. Warming in recent years has increased MPB activity, including at high elevations where extensive mortality has occurred in whitebark pine (*Pinus albicaulis*), lodgepole pine (*P. contorta*), and limber pine (*P. flexilis*) (Cleaver and others 2015, Macfarlane and others 2013). The relative vulnerability of other high-elevation pine species to MPB, however, remains unclear. Also unclear

is the role that evolved resinous defenses play in vulnerability of a particular pine species to MPB-caused mortality.

Pines have resinous secondary metabolite (SM) defenses, both constitutive and induced, that have evolved for protection against bark beetle attack, but they are metabolically expensive and divert resources from other processes including growth (Franceschi and others 2005). In general, constitutive defenses are considered reliable because they are always available. Induced defenses, in contrast, occur in response to an insect attack; therefore, costs are hypothesized to be only incurred as needed. Slow-growing and longer lived conifer species with high costs for tissue replacement are hypothesized to invest more in constitutive defenses because of their reliability, and investment in both constitutive and induced responses is considered redundant (Cipollini and others 2014). In a recent study, MPB attacks on the high-elevation Great Basin (GB) bristlecone pine (*P. longaeva*) were rare despite extensive mortality of adjacent limber pine. Similarly, in mixed stands of foxtail pine (*P. balfouriana*) and limber pine, attacks on foxtail pine were low despite high MPB-caused mortality of limber pine. In both species with rare or low preference by MPB, constitutive SM defense concentrations were up to eight times that of concentrations in co-occurring limber pine (Bentz and others 2017). These results suggest a level of protection from high constitutive SM concentrations, although induced responses have not been investigated in these species.

CHAPTER 10. Vulnerability of Rocky Mountain Bristlecone Pine to Mountain Pine Beetle

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Great Basin bristlecone and foxtail pine are long-lived species, and together with Rocky Mountain (RM) bristlecone pine (*P. aristata*), they make up the bristlecone or foxtail pine group (Lanner 2007). Although MPB attacks have been reported in RM bristlecone (Klutsch and others 2011), the vulnerability and defense capacity of this species, relative to its long-lived cousins, remains unclear. Our objectives were to (1) quantify MPB-caused mortality within the past 10 years in stands where RM bristlecone and limber pines co-occur, (2) evaluate in the field if MPB is capable of successful brood production in RM bristlecone pine, and (3) quantify the timing and concentration of constitutive and induced SM defenses, and morphological defenses, in RM bristlecone and limber pine growing in the same stands across the range of RM bristlecone pine.

METHODS

Location of mixed RM bristlecone and limber pine stands were determined using several sources including the U.S. Department of Agriculture Forest Service, Forest Inventory and Analysis (FIA) Database (USDA Forest Service 2017a), Forest Service District and U.S. Department of the Interior National Park Service vegetation maps, and personal communication. Tree mortality surveys were conducted in 13 stands throughout the Colorado portion of the RM bristlecone pine range where MPB activity had been noted in Insect and Disease Surveys (USDA Forest Service 2017b) or FIA plots within the past 10 years (fig. 10.1). Sampling for constitutive and induced resin characteristics

of the species was conducted at three sites in Colorado and two additional sites at the southern edge of the RM bristlecone pine distribution in Arizona and New Mexico (fig. 10.1). To quantify relative MPB preference for RM bristlecone pine and other co-occurring pines growing in the same stands, we used contiguous 405-m² fixed-radius circular plots extending linearly along a randomly chosen azimuth for as far as MPB-infested pine continued within the location. Live trees of all species were measured for diameter at breast height (d.b.h.; 7.5-cm minimum). Pines were examined for status (live, MPB-killed, or other mortality) and for signs of dwarf mistletoe (*Arceuthobium* spp.) and white pine blister rust caused by the nonnative pathogen *Cronartium ribicola*. Mountain pine beetle-killed trees were confirmed by the presence of egg galleries typical for MPB. For pines attacked by MPB, year of attack can be estimated for up to 3 years post-attack using characters of foliage color and needle retention. Basal area (BA) of live and dead trees, by species, was calculated (see Bentz and others 2021).

Defense measurements were taken on 12 RM bristlecone and 12 limber pines at each of 5 sites (see Soderberg and others, in preparation). At one site, co-occurring lodgepole pines were also sampled. Constitutive SM defenses were measured by taking a 1.8- x 6-mm phloem plug at d.b.h. from six equidistant locations around the circumference of the bole of each sampled tree. The removed tissue was placed in a vial, sealed, immediately placed on dry ice for transport, and stored at -40 °C until processing.

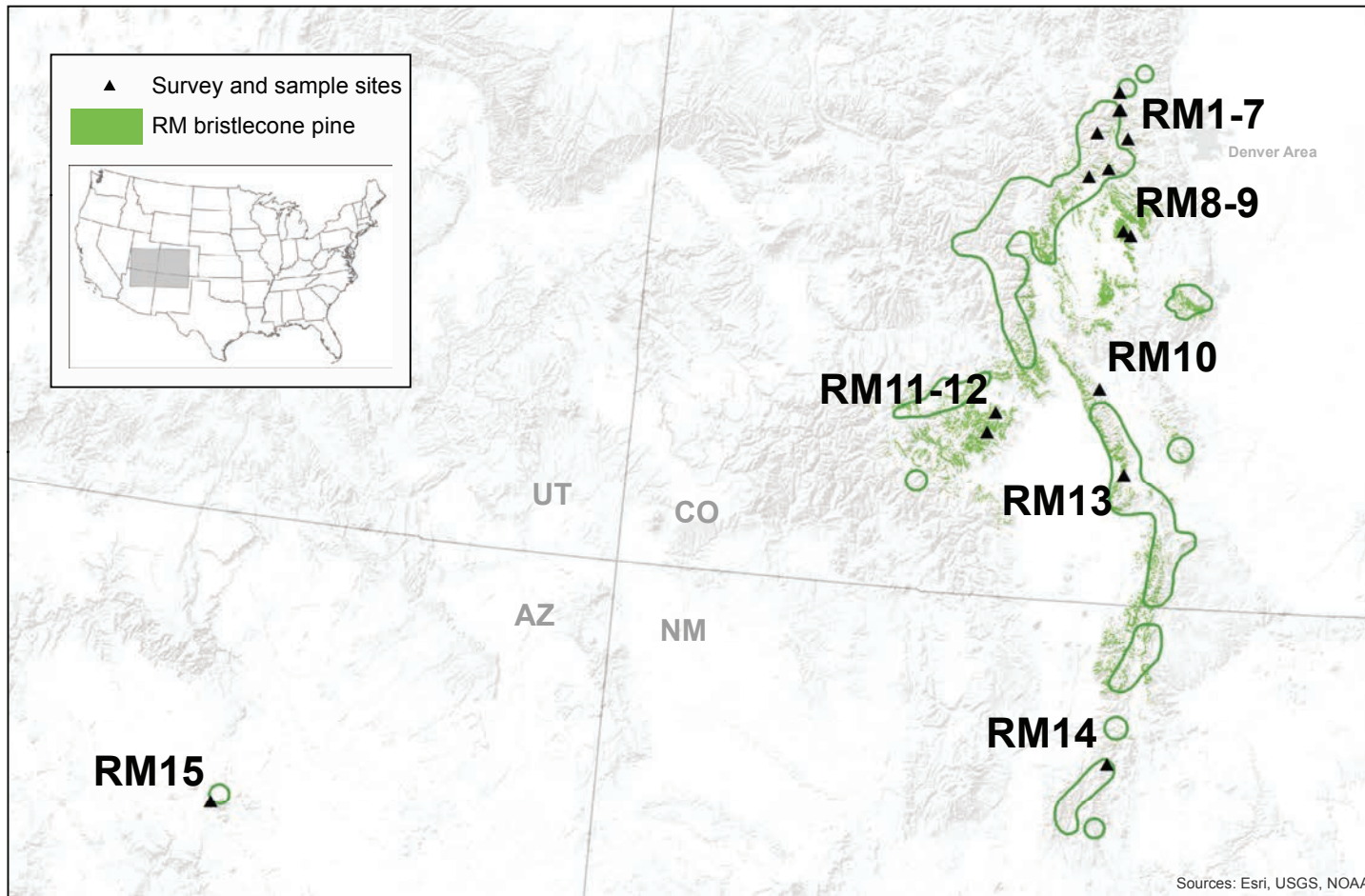


Figure 10.1—Location of Rocky Mountain (RM) bristlecone pine mortality survey (RM 1–13) and defense sample (RM 5, 9, 13, 14, 15) sites. Mortality surveys were conducted in 2017 and defense sampling in 2018 and 2019. Rocky Mountain bristlecone pine distribution based on Little (1971) and Ellenwood and others (2015).

To measure an induced response, the 12 constitutive sample trees were randomly chosen to receive either a mechanical wound (n = 6) or a wound plus fungal inoculant to simulate an MPB attack (n = 6). For the latter, we used a fungal inoculation of *Grosmannia clavigera*, a fungal symbiont of MPB that was placed into the 1.8- x 6-mm wound created by the constitutive sample. Inoculating trees with this MPB fungal associate has been shown to produce an induced response that differs from mechanical wounding alone (e.g., Keefover-Ring and others 2016). The timing, concentration, and composition of induced responses on each simulated attack and mechanical wounding tree were measured 1, 4, and 30 days after inoculation using a Trephor microtome sampling tool. Induced samples were transported and stored as indicated above for constitutive samples. Extracted phloem from constitutive, simulated attack and mechanical wounding sampling was analyzed using a coupled gas chromatography-mass spectrometry (GC-MS), and quantifications were made relative to an internal standard. Monoterpenoids, sesquiterpenoids, benzenoids, and a non-isoprenoid hydrocarbon were quantified and identified by comparison of chromatographic retention times and mass spectra with those of commercially available standards (Soderberg and others, in preparation). Only total SMs (sum of all compounds) are included in this report. A phloem sample and two cores per tree recording the preceding 10 years of growth were collected to analyze phloem and xylem resin ducts, and basal area increment (10-year BAI).

RESULTS AND DISCUSSION

Stands in the mortality surveys contained a mix of pine species, including RM bristlecone, limber, lodgepole, and ponderosa (*P. ponderosa*) pines, with RM bristlecone pine comprising a range of 4–92 percent of all pines at each site (table 10.1). No signs of white pine blister rust or dwarf mistletoe were observed on RM bristlecone pine within our plots. Based on galleries beneath the bark of dead trees, MPB was confirmed as the causal agent of death in all pine species surveyed. Emergence holes leading to pupal chambers on attacked trees also confirmed that MPB reproduced

Table 10.1—Stand conditions and metrics of mountain pine beetle (MPB)-killed pines at mortality survey sites (non-pines were a minor component)

Site	Species	Total pine percent	MPB- killed trees number	Basal area (live, pre- outbreak) m ² /ha	Basal area (MPB- killed) m ² /ha	d.b.h. (live, pre- outbreak) cm	d.b.h. (MPB- killed) cm
RM1	<i>P. aristata</i>	4.0	0	1.25	0.0	18.9	—
	<i>P. flexilis</i>	96.0	31	28.68	7.54	18.8	21.7
RM2	<i>P. aristata</i>	92.4	58	52.68	12.65	31.9	33.8
	<i>P. flexilis</i>	7.6	0	1.48	0.0	18.9	—
RM3	<i>P. aristata</i>	48.3	21	12.45	4.62	22.5	32.3
	<i>P. flexilis</i>	51.7	62	34.45	24.79	35.9	45.3
RM4	<i>P. aristata</i>	19.4	14	6.56	4.13	26.7	28.6
	<i>P. contorta</i>	80.6	17	15.09	3.54	18.3	24.2
RM5	<i>P. aristata</i>	35.7	1	6.01	0.37	22.5	28.7
	<i>P. contorta</i>	9.8	0	3.45	0.0	34.0	—
	<i>P. flexilis</i>	54.5	22	20.23	9.05	34.8	39.3

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Table 10.1 (continued)—Stand conditions and metrics of mountain pine beetle (MPB)-killed pines at mortality survey sites (non-pines were a minor component)

Site	Species	Total pine	MPB-killed trees	Basal area (live, pre-outbreak)	Basal area (MPB-killed)	d.b.h. (live, pre-outbreak)	d.b.h. (MPB-killed)
		percent	number	m ² /ha	m ² /ha	cm	cm
RM6	<i>P. aristata</i>	42.7	0	11.23	0.0	27.0	—
	<i>P. contorta</i>	57.3	5	14.51	1.90	25.7	32.4
RM7	<i>P. aristata</i>	81.9	37	21.43	11.67	20.9	27.9
	<i>P. contorta</i>	18.1	11	11.38	5.86	33.0	35.4
RM8	<i>P. aristata</i>	72.4	2	19.05	0.94	21.1	25.0
	<i>P. flexilis</i>	27.6	0	9.84	0.0	23.9	—
RM9	<i>P. aristata</i>	70.7	12	10.35	2.65	23.2	25.5
	<i>P. flexilis</i>	24.0	1	4.99	1.07	25.8	57.2
	<i>P. ponderosa</i>	5.3	0	1.35	0.0	31.8	—
RM10	<i>P. aristata</i>	10.5	0	1.58	0.0	20.4	—
	<i>P. contorta</i>	32.3	5	5.37	1.04	21.8	30.1
	<i>P. flexilis</i>	50.4	26	11.82	5.96	25.7	30.7
	<i>P. ponderosa</i>	6.8	2	2.19	1.41	28.8	56.0
RM11	<i>P. aristata</i>	88.9	10	10.16	2.34	19.6	34.4
	<i>P. contorta</i>	2.2	0	0.81	0.0	30.1	—
	<i>P. ponderosa</i>	8.9	3	2.55	1.16	31.7	46.5
RM12	<i>P. aristata</i>	90.9	2	10.50	1.62	24.0	33.5
	<i>P. contorta</i>	6.1	0	0.32	0.0	17.9	—
	<i>P. ponderosa</i>	3.0	0	0.38	0.0	27.9	—
RM13	<i>P. aristata</i>	59.9	24	12.97	3.60	22.8	32.5
	<i>P. flexilis</i>	36.9	10	17.74	2.10	31.6	39.1
	<i>P. ponderosa</i>	3.2	3	1.79	0.94	35.1	47.2
	<i>P. flexilis</i>	54.5	22	20.23	9.05	34.8	39.3

d.b.h. = diameter at breast height

— = no trees killed

successfully in RM bristlecone pine. We found that the proportion of each MPB-killed pine species at a site was correlated with host species availability. The proportion of a pine species killed by MPB over the 10-year period was best predicted by the proportion of BA of that species in the stand pre-outbreak ($F_{1,19} = 55.41$, $p < 0.0001$) (table 10.1, fig. 10.2). These results are similar to attack patterns found in mixed stands of hosts with known vulnerability to MPB including whitebark and lodgepole pine stands in Wyoming (Bentz and others 2015) and mixed lodgepole and ponderosa pine stands in Colorado (West and others 2014). Our results, however, are in contrast to surveys where the closely related GB bristlecone and foxtail pines grew in mixed stands with limber pine. Mountain pine beetle attacks were extensive on limber pine, low on foxtail pine, and rare on GB bristlecone pine regardless of the proportions of each species in the area (Bentz and others 2017). Rocky Mountain bristlecone pine appears to be more vulnerable to MPB attack than its close bristlecone relatives (Bentz and others 2021).

Across all sites, RM bristlecone pine was slower growing over the most recent 10 years than both limber and lodgepole pine. Rocky Mountain bristlecone also had greater concentrations of constitutive (day 0) SMs than both limber and lodgepole pines (fig. 10.3A). Similarly, the closely related and slower growing GB bristlecone and foxtail pines were also found to have greater constitutive SMs than co-occurring limber pine (Bentz and others 2017). Although simulated attack

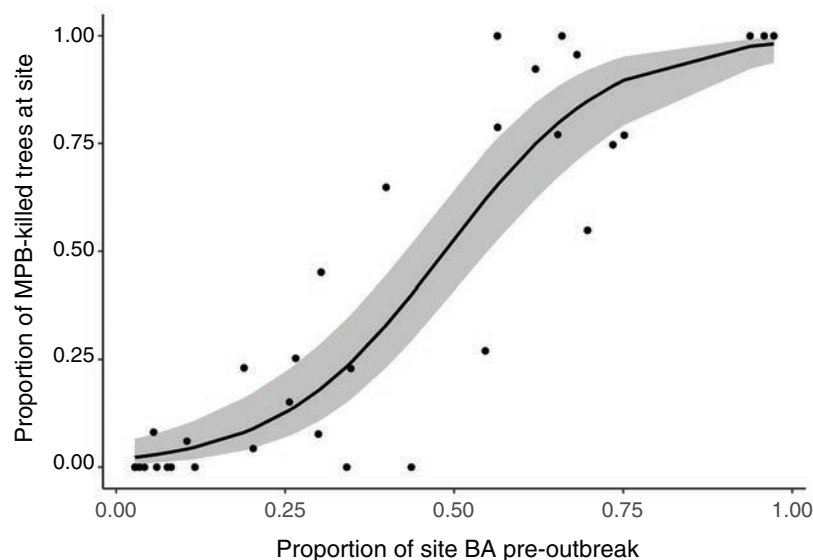


Figure 10.2—The proportion of a mountain pine beetle (MPB)-killed pine species at a site over a 10-year period (2006 to 2016) was best predicted by the proportion of basal area (BA) (m^2/ha) of that species in the stand pre-outbreak. Shown is the linear mixed model (SAS version 9.4, GLIMMIX) regression prediction (solid line) and the 95-percent confidence intervals (shaded area) for all species combined (Bentz and others 2021).

trees of RM bristlecone, limber, and lodgepole pine showed an induced increase in total SM compounds by day 4, the response was not different than mechanical wounding trees on that sample date. By day 30, however, the total response in simulated attack trees was >10x greater than day 0 (fig. 10.3D), and also greater than the day 30 mechanical wounding tree responses for all species and sites (Soderberg and others, in preparation). These results suggest

that response to a simulated attack did not occur rapidly (i.e., within 4 days). Although a large number of MPB attacks can occur within a few days (Bentz and others 1996), attacks can also be sustained over a month or longer (Bentz and others 2014). Our results suggest trees invest in induced defenses over a sustained period rather than a short period immediately following attack. In addition to having a greater level of constitutive SMs, RM bristlecone pine also had a greater overall induced response at day 30 than the response in limber pine. At the one site where RM bristlecone pine and lodgepole pine were sampled, however, lodgepole pine had a greater induced response on day 30. Our results suggest that investment in constitutive and induced defenses are not mutually exclusive, and in RM bristlecone pine, greater constitutive defenses were correlated with greater induced defenses (Soderberg and others, in preparation). Pines killed by MPB have been shown to invest less in resin duct-related defenses compared to trees that survived MPB attack (Hood and others 2015). In addition to xylem resin ducts, our ongoing analyses also include phloem resin ducts of the three species. Resin duct sampling included constitutive (day 0), 30 days, and 1 year post-induction.

Although RM bristlecone had much greater constitutive and induced defenses than limber pine, trees of both species were attacked, and attacks were most common on the species with the greatest frequency in the stand pre-outbreak. In addition to overall concentrations, composition of SM defenses may also influence

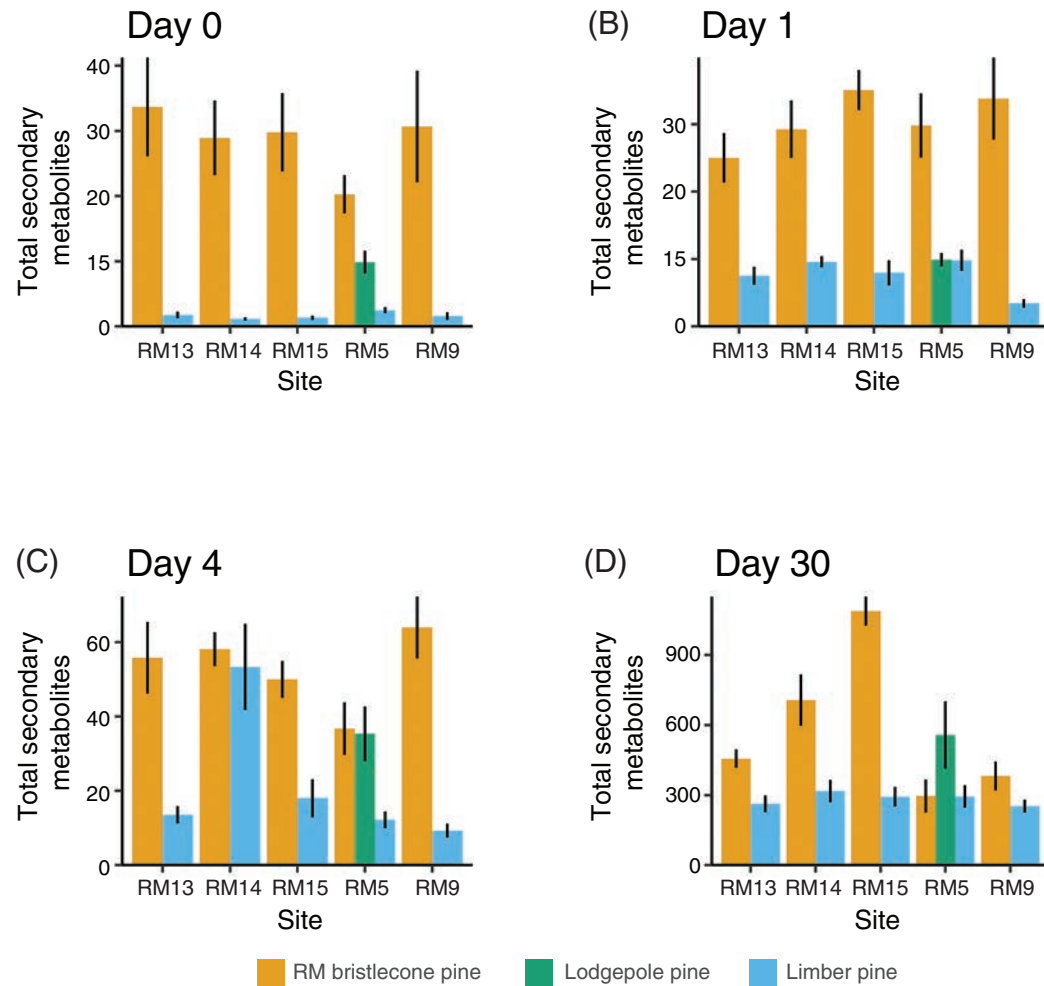


Figure 10.3—Total secondary metabolites (mg compound/g dry weight) in three pine species at five sites across the range of Rocky Mountain (RM) bristlecone pine (see fig. 10.1). Samples were collected at (A) day 0 (constitutive) and post-inoculation with a mountain pine beetle fungal associate (i.e., simulated attack) on (B) day 1, (C) day 4, and (D) day 30 (Soderberg and others, in preparation). Note the different y-axis scales among the days and that lodgepole pine was sampled at a single site.

MPB tree attack. Great Basin bristlecone and foxtail pine, which had rare to low attacks, contained >85-percent α -Pinene (- and + enantiomers combined) (Bentz and others 2017), and high levels of this terpenoid are known to be repellent to MPB as it can oxidize to verbenone (Lindgren and Miller 2002). In this study, RM bristlecone and limber pines had high proportions of δ -3-Carene, which can have both negative and positive roles in the attack process and survival of MPB and its associates (Boone and others 2013, Miller and Borden 2000).

Our results provide baseline data for building a foundation to better understand MPB responses to high-elevation pines and evolved tree defenses. More information is needed relating defense metrics of individual trees to successful versus unsuccessful attacks, in addition to knowledge of individual and ratios of compounds that influence MPB attack and reproductive success.

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